NANOSUSPENSIONS OF NOVEL DEUTERATED PYRAZOLOQUINOLINONES LIGAND (DK-I-56-1): LYOPHILIZATION PROCEDURE DEVELOPMENT THROUGH CRYOPROTECTANT SELECTION AND STABILITY STUDY

Jelena Mitrović, Maja Bjelošević, Jelena Đoković, Pegi Ahlin Grabnar, Odon Planinšek, Daniel Knutson, James Cook, Miroslav Savić, Snežana Savić
e-mail: snezana.savic@pharmacy.bg.ac.rs

ABSTRACT

Despite of good pharmacodynamics of DK-I-56-1, novel deuterated pyrazoloquinolinones ligand, low solubility limits its administration. Nanosuspensions can help to overcome this problem, but its small particle size usually leads to particle agglomeration in short period of time. This phenomenon can be prevented by performing lyophilization. In this study cryoprotectants selection as well as characterization (particle size measurements after redispersion, scanning electron microscopy, differential scanning calorimetry and thermogravimetric measurements) of obtained freeze dried preparations was carried out. It was observed that sucrose/mannitol ratio 1:1 and 3:2 in total concentration of 10% can preserve particle size during lyophilization. However, after stability study conducted during one month storage at 25 °C and 40 °C, particle size remained in submicron range only in one sample. Changes in particle size were also followed by changes in polymorphic form of mannitol. It can be concluded that changes of crystal forms in freeze dried preparations during storage could jeopardize their stability, and therefore should be carefully examined.

Keywords: nanosuspension, particle size, lyophilization, sucrose, mannitol.

INTRODUCTION

DK-I-56-1 (7-Methoxy-2-(4-methoxy-d3-phenyl)-2,5-dihydro-3H-pyrazolo[4,3-c]quinolin-3-one), has a potential therapeutic use in the treatment of depression, migraine and some neuropsychiatric disorders. Despite its good efficacy, low solubility hinder its administration. Nanosuspension formulation could be a promising tool to overcome these limitations. However, due to small particle size, nanocrystals tend to agglomerate during storage, which then compromises their application. By converting them to solid products it is possible to prevent agglomeration and increase stability. For this purpose, lyophilization is commonly used method. For formulation stabilization during lyophilization it is necessary to add cryoprotectants, and usually also bulking agent. The aim of this research was to investigate different ratios of sucrose and mannitol in lyophilization of nanosuspensions with respect to particle size preservation and acceptable cake appearance.

RESEARCH CONCEPT

Nanocrystal dispersions stabilized by polysorbate 80 (NS1) or combination of polysorbate 80 and poloxamer 407 (NS2) were prepared by wet ball milling. After addition of mannitol, sucrose or sucrose and mannitol in different ratios, samples were frozen and then freeze dried for 24h. Samples were characterized after lyophilization and after one month storage at 25 °C or 40 °C. Particle size was measured using Zetasizer Nano ZS (Malvern Instruments, UK) and Mastersizer (Malvern Mastersizer 2000 equipped with a small volume dispersion cell, Malvern, UK). The morphology of samples was investigated using field emission scanning electron microscopy (SEM; Zeiss Ultra Plus, Carl Zeis GmbH, Germany). Differential scanning calorimetry (DSC) (DSC1; Mettler Toledo, Switzerland) measurements were conducted in order to determine Tg' and Tg values. To determine residual water content, thermogravimetric measurements (TGA) (TGA/DSC 1 Mettler Toledo, Switzerland) were performed.

RESULTS AND DISCUSSION

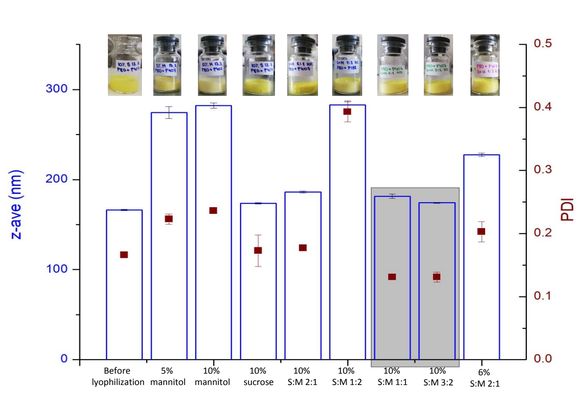


Figure 1 – Particle size (z-ave) and polydispersity index (PDI) of freeze dried samples after reconstitution (S-sucrose, M-mannitol).

In the first phase of experiments optimal cryoprotectants and their concentration was selected. Small size increase after redispersion was observed in samples with 10% sucrose, and sucrose/ mannitol ratio 2:1, 1:1 and 3:2, compared to other samples in which agglomeration was evident (Figure 1). Because of poor cake appearance of samples with 10% sucrose and sucrose:mannitol ratio 2:1, samples with sucrose:mannitol 1:1 and 3:2 were chosen for further analysis.

Particle size of chosen samples after lyophilization and subsequent redispersion increased for 50 nm (NS1) or 10 nm (NS2), and did not change significantly after 24h (Figure 2). After one month storage at 25 °C and 40 °C one sample (freeze dried NS1 with sucrose/mannitol 1:1, kept at 25 °C) remained stable regarding particle size, while agglomeration occurred in all other samples. This fenomenon was characterized by around fourfold particle size increase and additional peak > 1 μm in Mastersizer results.

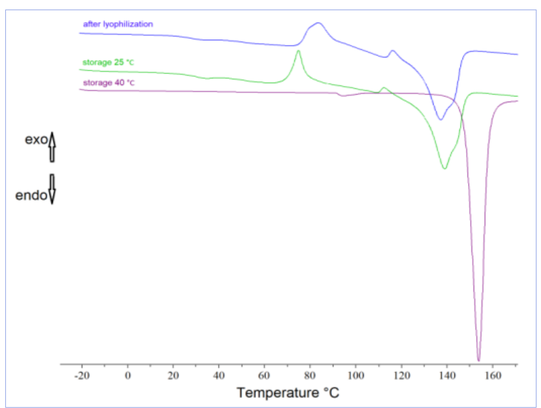
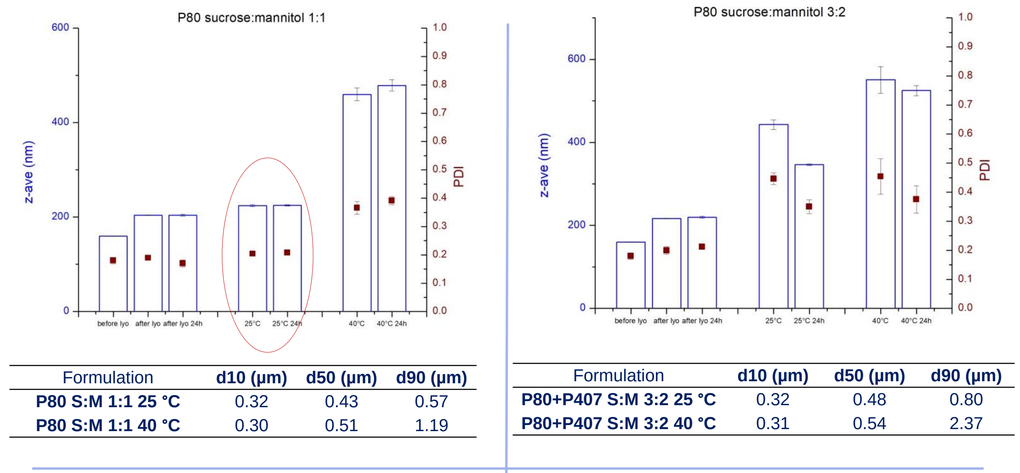


Figure 3 – DSC thermogram of freeze dried samples of NS1 with sucrose/mannitol 1:1 after lyophilization and after one month storage at 25 °C and 40 °C.

DSC curves analysis showed that Tg' (glass transition temperature of the maximally freeze-concentrated solution) of all samples (including placebo) was around -39 °C, while on DSC curves of solid samples exotermic peak at around 80 °C and endotermic peak at 134-139 °C were visible. On DSC curves of samples stored at 25 °C and 40 °C, no exotermic peak was present, and endotermic peak was shifted to higher temperatures (152 °C). DSC profile of one sample (NS1 with sucrose/mannitol ratio 1:1 kept at 25 °C), did not change during observed period (Figure 3). Change in DSC curve could be explained by polymorphic change of mannitol during storage, probably to δ form.

TGA measurements indicated low moisture content, from 0.1-0.3%, in all samples.

Because of small amount of active substance in samples, it was difficult to visualize nanocrystals on SEM images. However, it was noticed that particles form aggregates, which were not homogenously spread in excipients (Figure 4).



Formulation	d10 (μm)	d50 (μm)	d90 (μm)
P80 S:M 1:1 25 °C	0.32	0.43	0.57
P80 S:M 1:1 40 °C	0.30	0.51	1.19

Formulation	d10 (μm)	d50 (μm)	d90 (μm)
P80+P407 S:M 3:2 25 °C	0.32	0.48	0.80
P80+P407 S:M 3:2 40 °C	0.31	0.54	2.37

Formulation	d10 (μm)	d50 (μm)	d90 (μm)
P80+P407 S:M 1:1 25 °C	0.33	0.47	0.74
P80+P407 S:M 1:1 40 °C	0.31	0.53	2.09

Formulation	d10 (μm)	d50 (μm)	d90 (μm)
P80+P407 S:M 3:2 25 °C	0.32	0.51	1.23
P80+P407 S:M 3:2 40 °C	0.31	0.57	2.47

Figure 2 – Particle size (z-ave), polydispersity index (PDI) and Mastersizer results (d10, d50 and d90) of freeze dried samples before lyophilization and after reconstitution and storage at 25 °C and 40 °C.

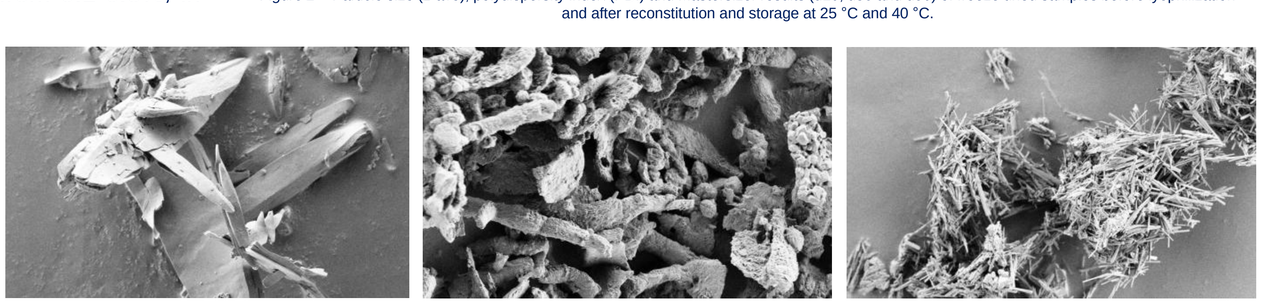


Figure 4 – SEM images of unprocessed DK-I-56-1 (left), freeze dried sample of NS2 without cryoprotectants (middle) and freeze dried sample of NS2 with sucrose/mannitol 1:1 after one month storage at 25 °C (right).

CONCLUSION

Lyophilization could be a promising method for improvement of DK-I-56-1 nanosuspensions stability. By selecting proper cryoprotectant and bulking agent ratios and concentrations, freeze dried products with satisfying properties could be obtained. However, mannitol polymorphic changes might jeopardize particle size preservation over time. For this reason, despite of good results after lyophilization, changes that might occur during storage must be taken into account and examined carefully. Different freeze drying conditions, cryoprotectant choice and storage conditions may help to overcome particle growth and agglomeration.

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